# SUMMARY

The electrochemical proton-gradient ($\Delta\tilde{\mu}_{H^+}$) plays a central role in bacterial metabolism. A $\Delta\tilde{\mu}_{H^+}$ can be generated by the translocation of protons from the cytoplasm to the external medium, as a result of electron transport in membrane-bound electron transfer chains and/or ATP hydrolysis by the membrane-bound ATPase. Since protons are positively charged, proton extrusion results in the generation of both a pH-gradient ($\Delta$pH) and a membrane potential ($\Delta\psi$): $\Delta\tilde{\mu}_{H^+} = \Delta\psi - Z\Delta pH$. The electrochemical proton gradient is a driving force and a regulatory parameter in various cellular processes, such as motility, ATP synthesis and the transport of solutes across the cytoplasmic membrane.

Anaerobic bacteria which do not possess electrontransfer chains seem to be able of $\Delta\tilde{\mu}_{H^+}$ generation by ATP hydrolysis only. This could be a problem, since in these bacteria usually only very little ATP is produced during breakdown of the energy source. This problem could be partly solved, if the excretion of the metabolic endproducts would supply the cell with additional energy. The transport of various solutes across the membrane is mediated by specific membrane bound proteins, the carriers. The $\Delta\tilde{\mu}_{H^+}$ is used in many transport systems to transport solutes across the membrane, since concomittant with this transport also proton- and/or charge translocation occurs. The energy present in $\Delta\tilde{\mu}_{H^+}$ is then converted in energy of solute gradients. On the other hand, translocation of solutes in the opposite direction results of course in the generation of a $\Delta\tilde{\mu}_{H^+}$, and this is what led to the postulation of the "energy-recycling" model.

The basic assumption of the "energy-recycling" model is that during carrier-mediated excretion of metabolic endproducts also protons and/or postive charges are excreted. Endproduct excretion then results in $\Delta\tilde{\mu}_{H^+}$ generation and therefore can contribute to the metabolic energy requirement of the bacteria. In this thesis the excretion of the metabolic endproduct lactate is studied in experiments with membrane vesicles of Escherichia coli and intact cells of Streptococcus cremoris. The artificial

creation of an outwardly directed lactate concentration gradient in E. coli vesicles results at pH 6.6 in the generation of a membrane potential $\Delta\psi$ (inside negative) and can drive the uptake of the amino acid proline. Both processes are completely inhibited by uncoupler (a $H^+$-ionophore), indicating that lactate efflux results in proton extrusion. Similar experiments in S. cremoris lead to the same conclusion: lactate excretion results in $\Delta\tilde{\mu}_{H^+}$ generation. In addition it is shown that in growing cells of S. cremoris indeed always an outwardly directed lactate gradient is present.

Of course it is very important to know the number of protons that are translocated together with one lactate anion, since this ratio, the $H^+$/lactate stoichiometry (n), determines the magnitude of the metabolic energy production by lactate excretion. In E. coli vesicles the value of n was determined from lactate uptake experiments: n is dependent on the external pH and varies between 1 (at pH 5.5) and 2 (at pH 8.0). The $H^+$/lactate stoichiometry has also been determined in growing and glycolyzing cells of S. cremoris. Assuming that in these cells the driving force for lactate translocation is very close to zero, n can be calculated from the data on $\Delta\psi$ , $\Delta$pH and the lactate gradient ($\Delta\bar{\mu}_{lac}$): $n = (\Delta\psi - \Delta\bar{\mu}_{lac})/\Delta\tilde{\mu}_{H^+}$. The value of n appears to be dependent on both the external pH and the lactate concentration; decreasing the pH and/or increasing the external lactate concentration results in a lower n in the S. cremoris cells. $H^+$/lactate stoichiometries between 1.8 (pH 7.0, 2 mM lactate) and 0.7 (pH 5.5, 50 mM lactate) have been calculated.

In Streptococcus cremoris the metabolic energy (in ATP-equivalents) generated by lactate excretion can be calculated if n is known. During sugar fermentation per lactate anion also one proton is produced internally and 1 ATP is synthesized. Lactate is excreted together with n protons, the production and excretion of one molecule of lactate will therefore result in the excretion of (n-1) protons. Since 2 protons are excreted per ATP hydrolyzed, lactate excretion will supply (n-1)/2 ATP equivalents per lactate. The energy gain will be 50% if n equals 2, whereas for n=1 no energy is gained by lactate excretion. A higher energy generation during sugar fermentation would of course result in a higher cell yield. This was observed in

continuous cultures of S. cremoris: at pH 7.0 the cell yield was about 12% higher than at pH 5.7, which can be explained by the effect of pH on n and therefore on the contribution of the lactate excretion process to the energy generation.

In conclusion it can be said that this thesis clearly demonstrates that the carrier-mediated excretion of metabolic end-products such as lactate can result in $\Delta\tilde{\mu}_{H^+}$ generation and can contribute to the energy requirements of bacteria, as was postulated in the "energy-recycling" model.